Arginase as a potential target in the treatment of cardiovascular disease: reversal of arginine steal?

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Received 2 November 2012; revised 4 February 2013; accepted 5 February 2013; online publish-ahead-of-print 14 February 2013

Abstract

Functional integrity of the vascular endothelium is of fundamental importance for normal vascular function. A key factor regulating endothelial function is the bioavailability of nitric oxide (NO). Recently, the enzyme arginase has emerged as an important regulator of NO production by competing for l-arginine, which is a substrate for both arginase and NO synthase. Increased activity of arginase may reduce the availability of l-arginine for NO synthase, thus reducing NO production, increasing formation of reactive oxygen species, and leading ultimately to endothelial dysfunction. Increased activity and expression of arginase have been demonstrated in several pathological cardiovascular conditions, including hypertension, pulmonary arterial hypertension, atherosclerosis, myocardial ischaemia, congestive heart failure, and vascular dysfunction in diabetes mellitus. Experimental studies have demonstrated that inhibition of arginase under these conditions increases NO bioavailability, reduces oxidative stress, improves vascular function, and protects against ischaemia–reperfusion injury. Initial clinical interventional studies are also promising. The purpose of this review is to discuss the role of arginase in cardiovascular pathologies, its contribution to the development of several cardiovascular disease states and the feasibility of using arginase inhibition as a therapeutic strategy.

Keywords

Arginase • Nitric oxide • Reactive oxygen species • Atherosclerosis • Ischaemia • Diabetes mellitus • Hypertension

1. Introduction

The vascular endothelium plays a key role in the maintenance of normal vascular function by modulating vascular tone, inflammation, and homeostasis. Conversely, endothelial dysfunction is observed early in the development of cardiovascular disease and is considered to be of pathophysiological importance in several cardiovascular diseases including hypertension, atherosclerosis, and vascular complications in diabetes mellitus. 1 Although the underlying cause of endothelial dysfunction is multifactorial, a key mechanism is considered to be impairment of the bioavailability of nitric oxide (NO). 2 which is defined as reduced biological activity due to reduced production and/or increased inactivation of endothelium-derived NO.

NO is produced from the amino acid l-arginine by endothelial NO synthase (eNOS). Interestingly, l-arginine is also a substrate for arginase, which converts l-arginine to l-ornithine and urea. 3 This means that the production of NO is dependent on the relative expression and activities of arginase and eNOS. More specifically, increased arginase activity may lead to deficiency of l-arginine available for eNOS and thereby reduce NO production. This has emerged as an important mechanism behind impaired NO bioavailability and endothelial dysfunction. The purpose of this review is to discuss the role of arginase in the development cardiovascular pathologies with special emphasis on its interaction with eNOS-derived NO, and the potential use of arginase inhibition as a novel therapeutic strategy in these conditions.

2. Localization and regulation of arginase

Arginase is a manganese metalloenzyme that hydrolyses l-arginine to urea and l-ornithine. Arginase exists in two distinct isoforms, arginase I and II, that share ~60% sequence homology. 4 Although both isoforms are found throughout the body, arginase I is a cytosolic enzyme mainly localized in the liver. Hepatic arginase I contributes most of the body’s total arginase activity and has a pivotal role in eliminating nitrogen formed during amino acid and nucleotide metabolism via the urea cycle. More recently, arginase I expression has been demonstrated in extra-hepatic tissues including endothelial cells and vascular smooth muscle cells. Arginase II is a mitochondrial enzyme with a wide distribution and is expressed in the kidney, prostate, gastrointestinal tract, and the vasculature. The role of arginase II is not completely understood, but the enzyme is assumed to be...
involved in the regulation of L-arginine homeostasis and production of L-ornithine for polyamine and proline synthesis for cell proliferation and development. Both isoforms are expressed in the vasculature, but it appears as if the expression is both vessel and species dependent. The expression of the different arginase isoforms in different cell types and species is summarized in Table 1.

Expression of arginase is stimulated by a variety of pro-inflammatory factors, including lipopolysaccharide, tumour necrosis factor (TNF)-α, and interferon-γ. Interleukin (IL)-4, IL-10, and IL-13 induce expression of arginase in macrophages. Additional stimuli for arginase expression are oxidised (ox) LDL, glucose, thrombin, hypoxia, and angiotensin II. Reactive oxygen and nitrogen species including H₂O₂ and peroxynitrite derived from eNOS and NADPH (nicotinamide adenine dinucleotide phosphate) oxidase stimulate arginase expression. Intracellular signalling pathways activated by these factors are the protein kinase C/RhoA/Rho kinase (ROCK) pathway, mitogen-activated protein kinase, tyrosine kinases, and cyclic adenosine monophosphate/protein kinase A. These signalling pathways are schematically summarized in Figure 1. Transcription factors regulating arginase expression include STAT-6, C/EBPβ, PU1, and PPARγ and δ.

Arginase activity can be modulated independently of changes in the levels of the arginase protein itself. Arginase I undergoes post-translational S-nitrosylation that stabilizes the arginase trimer, decreasing the Km for L-arginine by a factor of 6. This effect is observed in endothelial cells and is suggested to be mediated by NO produced by the inducible isoform of NOS (iNOS). An additional mechanism increasing arginase II activity is a receptor-mediated activation of RhoA and ROCK resulting in subcellular redistribution of arginase II from the mitochondria and microtubule cytoskeleton. Such an effect may explain the rapid increase in arginase activity (within 5 min) induced by oxLDL in endothelial cells, long before any increase in protein expression can be expected. Other post-translational modifications such as phosphorylation are currently not described.

### Table 1  Cellular expression of arginase

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Isoform</th>
<th>Species/region/cell</th>
<th>Comment</th>
<th>Reference</th>
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<tr>
<td>Endothelial cell</td>
<td>Arg I</td>
<td>Human coronary</td>
<td>Increase in DM</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pig coronary</td>
<td>Increase by I/R and H₂O₂</td>
<td>16,49,51</td>
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<tr>
<td></td>
<td></td>
<td>Mouse coronary</td>
<td>Increase by I/R</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BCEC</td>
<td>Increase by high glucose</td>
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<tr>
<td></td>
<td>Arg II</td>
<td>HAEC</td>
<td>Increase in HT</td>
<td>103</td>
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<tr>
<td></td>
<td></td>
<td>Rat mesenteric</td>
<td>Controls and diabetic</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pig coronary</td>
<td></td>
<td>64</td>
</tr>
<tr>
<td>Vascular smooth muscle</td>
<td>Arg I</td>
<td>Pig coronary</td>
<td>Increased by oscillatory shear stress</td>
<td>105</td>
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<tr>
<td></td>
<td></td>
<td>Human aortic</td>
<td>Increased by I/R</td>
<td>43</td>
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<tr>
<td></td>
<td>Arg II</td>
<td>Pig coronary</td>
<td>Controls and diabetic</td>
<td>64</td>
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<tr>
<td>Cardiomyocyte</td>
<td>Arg I</td>
<td>Rat coronary</td>
<td>Increased in DM</td>
<td>64</td>
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<tr>
<td></td>
<td></td>
<td>Rat mesenteric</td>
<td>Unchanged in HT</td>
<td>103</td>
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<tr>
<td></td>
<td>Arg II</td>
<td>Pig</td>
<td></td>
<td>51</td>
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<td>Cat</td>
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<tr>
<td>Macrophage</td>
<td>Arg I</td>
<td>Mouse bone marrow-derived (M2)</td>
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<td></td>
<td>Arg II</td>
<td>Mouse bone marrow-derived (M1)</td>
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<td>PMN</td>
<td>Arg I</td>
<td>Human</td>
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<td>51,106</td>
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<tr>
<td></td>
<td>Arg II</td>
<td>Pig</td>
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<td>51</td>
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</tbody>
</table>

Arg, arginase; BCEC, bovine coronary endothelial cells; DM, diabetes mellitus; HAEC, human aortic endothelial cells; HT, hypertension; HUVEC, human umbilical vein endothelial cells; I/R, ischemia/reperfusion; PMN, polymorphonuclear neutrophils.

### 3. Arginase, NO production, and eNOS

The fact that arginase and eNOS utilize L-arginine as their common substrate results in important reciprocal interactions between these enzymes. As illustrated in Figure 2, an increase in arginase activity may lead to consumption of L-arginine needed for NO production by eNOS and hence to endothelial dysfunction. Considering that reduced bioavailability of NO and endothelial dysfunction are critically involved in the development of several cardiovascular disorders, up-regulation of arginase activity may be an important underlying cause. It should be noted that although L-arginine also is the substrate...
for the enzymes arginine:glycine amidinotransferase and arginine de-carboxylase producing creatine and agmatine, respectively, arginase and NOS are the arginine catabolic enzymes with the most impact on the cardiovascular system.5

Several studies have convincingly demonstrated that increased arginase activity is associated with endothelial dysfunction. This is shown in various experimental models of hypertension,25 atherosclerosis,10 diabetes,11 and ageing.26 The effect appears to be due to impaired production of NO secondary to L-arginine deficiency. An important contributing factor is so-called uncoupling of eNOS, a situation when the enzyme produces superoxide rather than NO as a result of substrate and/or co-factor deficiency.27 Thus, inhibition of arginase increases the bioavailability of NO and reduces the levels of superoxide11,28 resulting in improved endothelial function (Figure 2). In addition, increased cytosolic arginase II during hypoxia is co-localized with eNOS.13 The close proximity of the two enzymes that share L-arginine as their substrate hints at an intriguing mechanism for control of NO synthesis. Finally, arginase may also inhibit L-arginine transport in endothelial cells further reducing substrate availability for eNOS.13 The role of arginase in the development of cardiovascular disease has therefore been investigated in various experimental models and initial clinical studies using specific arginase inhibitors. These studies are summarized in Table 2 and described below.

![Figure 1](https://academic.oup.com/cardiovascres/article-abstract/98/3/334/390065)

Figure 1 Schematic illustration of factors regulating arginase expression and activity. Various factors including cytokines, oxidized LDL, angiotensin II, reactive oxygen, and nitrogen species activate different intracellular signalling pathways. cAMP, cyclic adenosine monophosphate; AII, angiotensin II; LDL, low-density lipoprotein; MAPK, mitogen-activated protein kinase; ONOO−, peroxynitrite; PKA, protein kinase A; PLC, protein kinase C; ROCK, Rho kinase; TNF, tumour necrosis factor.

![Figure 2](https://academic.oup.com/cardiovascres/article-abstract/98/3/334/390065)

Figure 2 Schematic illustration of the action of arginase in the regulation of NO bioavailability and vascular function. Arginase is expressed in endothelial and vascular smooth muscle cells via regulation of cytokines, thrombin, hypoxia, reactive oxygen species, hyperglycaemia, and oxidized LDL. Increased activity of arginase will via hydrolysis of L-arginine to ornithine and urea reduce the availability of L-arginine for NO synthase (NOS), thereby reducing the production of NO. Lack of L-arginine will also result in ‘uncoupling’ of NOS whereby the enzyme produces superoxide instead of NO. Generation of superoxide by uncoupled eNOS and NADPH oxidase and peroxynitrite from superoxide and NO will further increase arginase activity and impair NO production via oxidation of tetrahydrobiopterin. Collectively, these changes will reduce the bioavailability of NO and contribute to endothelial dysfunction. In vascular smooth muscle cells, ornithine will increase formation of L-proline and polyamines which stimulate cell proliferation. Ang II, angiotensin II; BH4, tetrahydrobiopterin; LDL, low-density lipoprotein; LPS, lipopolysaccharide; NADPHox, nicotinamide adenine dinucleotide phosphate oxidase; NO, nitric oxide; ONOO−, peroxynitrite; VSMC, vascular smooth muscle cell.
Arginase in cardiovascular disease

Table 2 Interventional studies with arginase inhibitors in experimental and clinical studies

<table>
<thead>
<tr>
<th>Model/patients</th>
<th>Intervention</th>
<th>Main effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atherosclerosis/coronary artery disease</td>
<td>Chronic infusion of BEC by osmotic minipump</td>
<td>Improvement in endothelial function, reduction in superoxide production, reduction in atherosclerosis</td>
<td>31</td>
</tr>
<tr>
<td>ApoE&lt;sup&gt;-/-&lt;/sup&gt; mice</td>
<td>Chronic oral ABH administration</td>
<td></td>
<td></td>
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<tr>
<td>Patients with coronary artery disease</td>
<td>Iv infusion of nor-NOHA</td>
<td>Improvement in forearm endothelial function.</td>
<td>70</td>
</tr>
<tr>
<td>Myocardial I/R</td>
<td>Iv nor-NOHA before ischemia</td>
<td>Reduction in infarct size</td>
<td>50</td>
</tr>
<tr>
<td>Rat &lt;i&gt;in vivo&lt;/i&gt;</td>
<td>Iv nor-NOHA before reperfusion</td>
<td>Reduction in infarct size</td>
<td>51</td>
</tr>
<tr>
<td>Pig &lt;i&gt;in vivo&lt;/i&gt;</td>
<td>DFMO and nor-NOHA ex vivo</td>
<td>Improvement in endothelial function</td>
<td>49</td>
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<tr>
<td>Mouse ex vivo</td>
<td>Nor-NOHA ex vivo</td>
<td>Improvement in endothelial function</td>
<td>52</td>
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<tr>
<td>Diabetes mellitus</td>
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<tr>
<td>Rat type 1 diabetes</td>
<td>DFMO ex vivo</td>
<td>Improvement in endothelial function</td>
<td>11</td>
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<tr>
<td>Mouse type 1 diabetes</td>
<td>BEC ex vivo</td>
<td>Improvement in endothelial function</td>
<td>62</td>
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<tr>
<td>Rats</td>
<td>ABH sc for 5 days</td>
<td>Improvement in retinal endothelial function</td>
<td>68</td>
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<tr>
<td>Pig (aortic coarctation)</td>
<td>Nor-NOHA iv</td>
<td>Improved myocardial microvascular function</td>
<td>64</td>
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<tr>
<td>Patients with type 1 and type 2 diabetes</td>
<td>Nor-NOHA ex vivo</td>
<td>Improvement in coronary arterial endothelial function</td>
<td>69</td>
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<tr>
<td>Patients with type 2 diabetes and coronary artery disease</td>
<td>Iv infusion of nor-NOHA</td>
<td>Improvement in forearm endothelial function.</td>
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<td>Heart failure</td>
<td>Topical application of nor-NOHA</td>
<td>Increase in sublingual microvascular flow</td>
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<td>Hypertension</td>
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<tr>
<td>Pigs (aortic coarctation)</td>
<td>Nor-NOHA ex vivo</td>
<td>Improvement in endothelial function</td>
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<tr>
<td>Spontaneously hypertensive rats</td>
<td>Nor-NOHA ip for 3 weeks</td>
<td>Reduction in MAP, reduction in myogenic tone, improvement in endothelial function</td>
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<td>Patients with hypertension</td>
<td>BEC + nor-NOHA via cutaneous microdialysis</td>
<td>Increased reflex cutaneous vasodilatation</td>
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<tr>
<td>PAH</td>
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<tr>
<td>Rats with pulmonary embolism</td>
<td>Iv nor-NOHA</td>
<td>Reduced the rise in pulmonary vascular resistance in vivo and improved endothelial function ex vivo</td>
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<tr>
<td>Rats</td>
<td>BEC ex vivo</td>
<td>Improved endothelial function</td>
<td>23</td>
</tr>
<tr>
<td>Humans</td>
<td>BEC + nor-NOHA via cutaneous microdialysis</td>
<td>Increased reflex cutaneous vasodilatation</td>
<td>101</td>
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</table>

ABH, 2-(S)-amino-6-boronohexanoic acid; ApoE, apolipoprotein E; BEC, S-(2-boronoethyl)-L-cysteine; DFMO, α-difluoromethylornithine; Ia, intra-arterial; Ic, Intracoronar; Iv, intravenous; I/R, ischaemia-reperfusion; MAP, mean arterial pressure; nor-NOHA, N<sup>-</sup>-hydroxy-nor-L-arginine; PAH, pulmonary arterial hypertension.

4. Atherosclerosis

Endothelial dysfunction as a result of reduced bioavailability of NO is considered to be an early event in the development of atherosclerosis. Given that arginase competes with eNOS for their common substrate L-arginine, it is reasonable to assume that increased arginase activity is an important factor in the development of atherosclerosis. Several studies have demonstrated increased expression of the arginase protein and/or arginase activity in experimental models of atherosclerosis. Apolipoprotein E knockout (apoE<sup>-/-</sup>) mice fed a cholesterol-rich diet had significantly higher arginase activity in the aorta than age-matched wild-type mice. Arginase activity was reduced after the removal of the endothelium, suggesting important contribution from endothelial cells. Increased arginase activity was also found in apoE<sup>-/-</sup> mice fed a cholesterol-rich diet, and in atheromatous aortic lesions of hyperlipidemic rabbits. The predominant isoform of arginase in apoE<sup>-/-</sup> atherosclerotic mice seems to be arginase II. Species apparently differ in terms of arginase expression, however, since the expression of both arginase I and arginase II was increased in aorta of rabbits with atherosclerosis.

Various mechanisms regulating arginase activity seem to exist in atherosclerosis. Ming et al. demonstrated that the induction of arginase activity in endothelial cells was regulated via the RhoA/ROCK pathway. Interestingly, arginase activity was inhibited by the HMG-CoA reductase inhibitor lovastatin that inhibits RhoA via geranylation. OxLDL, the primary proatherogenic lipoprotein, induces activation of arginase via the endothelial lectin-like oxLDL scavenger receptor-1 (LOX-1) and RhoA/ROCK. Arginase activity in endothelial cells is increased by reactive oxygen species via a mechanism that also involves the RhoA/ROCK pathway. Arginase expression has also been detected in macrophages, which play a key role in atherosclerosis. Classical pro-inflammatory M1 macrophages express arginase II and alternatively activated anti-inflammatory M2 macrophages express arginase I in atherosclerotic lesions of apoE<sup>-/-</sup> mice. The expression of arginase in macrophages was induced by the cytokines IL-4 and IL-6, interferon-γ, and lipopolysaccharide.
Increased arginase activity attenuates NO production and contributes to endothelial dysfunction in atherosclerosis. Arginase inhibition restored NO production in human endothelial cells following exposure to oxLDL, increased NO production in aorta from apoE−/− mice, and improved endothelium-dependent dilatation of pig coronary arterioles exposed to oxLDL. Of additional interest are the observations that aortic endothelial cells from arginase II−/− mice had higher basal NO production than corresponding cells from wild-type mice, and oxLDL failed to reduce NO production in arginase II−/− mice. Endothelium-dependent vasorelaxation of aorta from apoE−/− mice was markedly enhanced by the arginase inhibitor BEC, suggesting that arginase is an important contributor to the attenuated endothelial function in atherosclerosis. Arginase inhibition also reduced superoxide production in aorta from apoE−/− mice via a NOS-sensitive pathway. These observations suggest that increased endothelial arginase activity not only contributes to impaired NO production but also to uncoupling of NOS. This effect may be due to L-arginine deficiency occurring in the presence of increased arginase activity. Reactive oxygen species will in turn increase arginase expression and activity, creating a vicious circle. Furthermore, arginase seems to contribute to reduced arterial compliance in the apoE−/− mouse in vivo. Finally—and of fundamental importance in atherosclerosis—chronic treatment with the arginase inhibitor BEC for 8 weeks reduced aortic plaque burden in apoE−/− mice.

Arginase may also promote vascular smooth muscle cell proliferation and extracellular matrix deposition via production of polyamines and L-proline, and thereby play a role in intimal hyperplasia and remodelling processes. Similar mechanisms may underlie the role of arginase in vascular growth after balloon injury of carotid arteries. Arterial injury stimulated arginase activity and arginase I protein expression in the vessel wall, and local arginase inhibition lead to a decline in smooth muscle cell synthesis and reduced intimal thickening. In addition, arginase I promoted the entry of smooth muscle cell into the cell cycle. It should also be pointed out, however, that arginase may in certain situations exert anti-inflammatory effects. Smooth muscle cells transfected with arginase I had reduced cytokine production in response to lipopolysaccharide, and arginase inhibition increased cytokine production. Furthermore, up-regulation of arginase I reduced macrophage infiltration and inflammation in atherosclerotic plaque in rabbits. In addition, macrophage-derived arginase I may contribute to resistance to atherosclerosis in rabbits. These observations indicate that arginase I inhibits inflammatory activity via interference with iNOS. The regulatory role of arginase I and arginase II for vascular function and inflammation in atherosclerosis is complex and critically dependent not only on the expression of the two isoforms of arginase but also on the expression of eNOS and iNOS. Further studies using isoform-specific inhibitors of arginase and NOS are therefore warranted to fully understand their roles in atherosclerosis.

5. Myocardial ischaemia and reperfusion

It was described early on that serum arginase activity was increased in patients with acute myocardial infarction and correlated with the extent of myocardial necrosis. Smirnov et al. demonstrated increased arginase activity in infarcted human myocardial tissue in comparison with normal myocardium. Interestingly, they found a positive veno-arterial concentration gradient of urea over the coronary vascular bed in patients with ischaemic heart disease, suggesting local production of urea from arginase.

The expression of arginase following ischaemia/reperfusion (I/R) has been investigated in various experimental models. Hein et al. demonstrated that the expression of arginase in coronary arterial endothelial cells and vascular smooth muscle cells was increased following I/R. Up-regulation of myocardial arginase I expression following I/R has also been demonstrated in rat in vivo. In a pig model of I/R arginase activity was increased and associated with expression of both isoforms in endothelial cells, vascular smooth muscle cells, cardiomyocytes, and polymorphonuclear neutrophils. In a mouse model of I/R, arginase I was not expressed in neutrophils within the reperfused myocardium, whereas its expression was increased in endothelial cells. Myocardial expression of arginase during I/R is thought to be regulated via the influence of cytokines. Gao et al. demonstrated that I/R-induced up-regulation of arginase I was absent in TNF−/− mice, and anti-TNF-α reduced arginase I expression in wild-type mice. Furthermore, arginase activity was reduced in TNF−/− mice.

Increased expression and activity of arginase seem to be of fundamental functional importance in myocardial I/R. I/R-induced attenuation of NO production and impairment of endothelium-dependent vasodilatation in coronary arteries was prevented following arginase inhibition. Jung et al. demonstrated that systemic arginase inhibition reduced infarct size by 51% in rats subjected to 30 min ischaemia and 2 h of reperfusion. In a pig model, in which local intracoronary infusion of the arginase inhibitor Nω-hydroxy-n-L-arginine (nor-NOHA) started 5 min before reperfusion, infarct size was similarly reduced by 50%. The cardioprotective effects could be blocked by inhibitors of NOS and by an NO scavenger. Interestingly, systemic arginase inhibition was associated with an increase in plasma citrulline and a reduction in plasma ornithine resulting in increase in plasma citrulline/ornithine ratio. There was also an increase in plasma and myocardial citrulline/arginine ratios. Collectively, these findings suggest that inhibition of arginase induces a shift in the utilization of arginine from arginase to NOS, resulting in increased NO production and protection against I/R injury. However, it is in this context important to note that arginase might be catalyzed by arginase via ornithine to polyamines that have been implicated in I/R injury. Especially spermine can exert protective effects in myocardial I/R. However, this effect seems to be related to increased NO production and the role of arginase activity in this setting remains to be elucidated.

6. Stroke

Although detailed experimental and clinical information regarding the role of arginase in ischaemic stroke is limited, there are data describing increased arginase activity following cerebral hypoxia–ischaemia. Gene expression profiling demonstrated an up-regulation of arginase I in peripheral white blood cells of patients with ischaemic stroke. These observations suggest arginase to be a biomarker but do not reveal information regarding its functional role in acute ischaemic stroke. Although experimental data from studies inhibiting arginase activity in stroke are lacking, there are indications that cerebral vascular function can be influenced by arginase. In an animal model of subarachnoid haemorrhage, increased arginase resulted in impaired availability of L-arginine and NO production.
The functional consequences of altered arginase expression and activity in diabetes have been investigated both in isolated vascular preparations and under in vivo conditions. Impaired endothelium-dependent vasorelaxation of coronary arteries from rats with type 1 diabetes was normalized by arginase inhibition. Recent findings suggest that aortic and retinal endothelial dysfunction in streptozotocin-induced type 1 diabetes is mediated by increased arginase I expression. However, there appear to be regional differences since the impairment of endothelium-dependent relaxation observed in the corpora cavernosa of type 1 diabetic mice was not seen in arginase II knockout mice, indicating that arginase II plays a role in this tissue. The role of arginase for vascular dysfunction in vivo was investigated in type 2 diabetic rats. Arginase inhibition improved myocardial microvascular dysfunction. The effect of arginase inhibition was blocked by NOS inhibition, suggesting that the effect was mediated through increased NO production (Figure 3). Furthermore, arginase inhibition was associated with increased citrulline/ornithine and citrulline/arginine ratios suggesting increased NOS activity. These observations suggest that arginase contributes to microvascular dysfunction in type 2 diabetes via a mechanism that is related to impaired bioavailability of NO.

There are some recent data supporting a role of arginase in vascular dysfunction in human diabetes. Coronary arterioles obtained from patients with diabetes displayed reduced endothelium-dependent relaxation in vitro and increased expression of arginase I in endothelial cells. Endothelium-dependent vasodilatation was increased by an arginase inhibitor. Furthermore, data from an in vivo study demonstrate that arginase inhibition markedly improves endothelium-dependent vasodilatation in the forearm of patients with type 2 diabetes and coronary artery disease (Figure 4) whereas it does not affect endothelial function in healthy controls. This observation indicates a functional role of arginase contributing to endothelial dysfunction in patients with diabetes.

8. Heart failure

There is strong agreement that low bioavailability of NO plays a pivotal role in heart failure patients due to increased plasma levels of the endogenous NOS inhibitor asymmetric dimethyl-L-arginine (ADMA). In addition, there is growing evidence that the induction of arginase activity plays an additional role in vascular dysfunction in
patients with heart failure. Increased plasma arginase activity in these patients may arise from enhanced spillover of enzyme from injured tissues such as a congested liver or damaged myocytes. Serum arginase concentration was decreased while cardiac arginase II expression was increased in a rabbit model of heart failure induced by left ventricular pacing for 3 weeks. Cat cardiomyocytes express arginase I that via reduction of NO bioavailability may regulate contractility. Increased cardiomyocyte arginase activity may, by attenuating NOS signalling, negatively influence heart failure. Combined administration of arginase and ADMA in rats decreased cardiac output and stroke volume. Conversely, different arginase inhibitors dose-dependently increased basal contractility in rat myocytes, an effect that was inhibited by both non-specific and specific neuronal NOS inhibitors. The authors conclude that arginase II negatively regulates neuronal NOS activity, most likely by limiting substrate availability in its microdomain. A recent clinical study demonstrated that plasma arginase I levels were significantly higher in patients with heart failure compared with controls. Patients with severe heart failure had significantly higher arginase I levels than patients with mild heart failure. Interestingly, local administration of an arginase inhibitor resulted in improved sublingual microcirculation by an NO-dependent mechanism. These initial clinical observations support a functional role of increased arginase activity in patients with heart failure.

9. Hypertension

The presence of endothelial dysfunction has been well documented in arterial hypertension and several studies support a mechanistic role of arginase. Increased arginase activity/expression was reported in various vascular beds in models of essential or secondary hypertension. Of further importance are the findings that arginase inhibition prevented the development of hypertension and improved aortic endothelial function via an NO-dependent mechanism when administered to prehypertensive, young or adult spontaneously hypertensive rats. Further intriguing data come from a study by Huynh et al. who reported on the cardiovascular phenotype of an arginase II−/− mouse. Although NO production was increased in the aorta and isolated endothelial cells from these mice and there was a trend towards increased response to acetylcholine, it was found that vascular sensitivity and reactivity altered over time and that an increased mean arterial pressure from 8 weeks of age was observed. This was associated with an increase in left ventricular weight, left ventricular systolic pressure, and diminished diastolic function. This surprising finding contradicts the a priori hypothesis that this knockout mouse would exhibit low blood pressure. The exact reason for the hypertensive phenotype remained elusive, and the authors speculate that central and renal regulation differences might account for these findings. This was supported by the data that showed that increased levels of NO in the central nervous system can set blood pressure levels to a higher level. Given that arginase II may reciprocally regulate NO bioavailability, it is possible that a lack of arginase II via increased NO levels results in a central resetting of blood pressure.

There are also indications of a role of arginase in human hypertension. Reflex cutaneous vasodilatation in patients with hypertension was augmented following administration of arginase inhibitors via skin microdialysis catheters. It was recently demonstrated that anti-hypertensive treatment with the angiotensin converting enzyme inhibitor lisinopril reduced erythrocyte arginase activity in patients with arterial hypertension. However, the functional role of erythrocyte arginase in vascular regulation remains unclear.

10. Pulmonary arterial hypertension

Pulmonary arterial hypertension (PAH) is characterized by increased vascular resistance due to pulmonary artery vasoconstriction, vascular remodelling, and thrombosis. Several molecular pathways and signalling molecules are altered in PAH, including the NO pathway. Owing to the aforementioned importance of arginase in the regulation of NO bioavailability, arginase is likely to be of pathophysiological relevance in PAH. Liver transplantation and reperfusion of the transplanted organ is associated with an acute release of hepatic arginase and pulmonary vasoconstriction. Conditions associated with chronically increased release of arginase from red blood cells, such as haemolytic disorders, have been linked to the development of PAH. In addition, PAH is also aetiologically associated with increased vascular arginase activity. Experimental pulmonary embolism leading to pulmonary hypertension is associated with increased pulmonary artery expression of arginase II, depletion of plasma L-arginine, and endothelial dysfunction. Arginase inhibition improved endothelium-dependent dilation of pulmonary artery rings. Another mechanism leading to PAH is hypoxia, which increases arginase II mRNA and protein expression as well as arginase activity in microvascular endothelial cells from human lung. In the same study, it was shown that inhibition of arginase by specific small interfering RNA or by a pharmacological inhibitor enhanced the production of NO. These findings are in line with a study demonstrating increased arginase II expression in pulmonary artery endothelial cells of patients with PAH. This leads to a depletion of L-arginine, which has been shown to be strongly correlated with right atrial pressure, cardiac...
old rats. Furthermore, arginase I knockdown with specific oligonucleotides restored NO production and endothelium-dependent relaxation of aorta from old rats. Another interesting observation is that cleotides restored NO production and endothelium-dependent relaxation of aorta from old rats. Another interesting observation is that impaired heat-induced cutaneous vasodilatation in old human subjects was improved following administration of arginase inhibitor via microdialysis. Although this observation suggests that arginase contributes to age-induced impairment of vascular function also in humans, it is difficult to establish with certainty that this difference is related to ageing and not to sub-clinical atherosclerotic disease in these aged subjects.

11. Ageing

Several observations indicate that arginase activity is up-regulated with increasing age and is involved in the development of endothelial dysfunction in ageing blood vessels of rats and mice, including atherosclerotic apoe−/− mice. This increase in activity appears to be attributed mainly to the arginase I isoform. In addition to increased arginase expression, there is evidence of increased arginase activity due to S-nitrosylation of arginase by iNOS. Arginase inhibition improved endothelial function and increased NO formation in old rats. Furthermore, arginase I knockdown with specific oligonucleotides restored NO production and endothelium-dependent relaxation of aorta from old rats. Another interesting observation is that impaired heat-induced cutaneous vasodilatation in old human subjects was improved following administration of arginase inhibitor via microdialysis. Although this observation suggests that arginase contributes to age-induced impairment of vascular function also in humans, it is difficult to establish with certainty that this difference is related to ageing and not to sub-clinical atherosclerotic disease in these aged subjects.

12. Therapeutic potential and future perspectives

As outlined above, arginase inhibition may potentially have beneficial effects in several pathological cardiovascular conditions. The therapeutic effect of arginase inhibition has been tested in a number of experimental models of cardiovascular disease with positive results. Data from clinical studies are very limited, however. Initial small-scale clinical ‘proof-of-concept’ studies involving local administration of arginase inhibitors via cutaneous microdialysis or intra-arterial infusion have yielded promising results regarding vascular function in patients with coronary artery disease and type 2 diabetes, heart failure, and hypertension. Since these observations suggest that up-regulated arginase activity is of importance also in human cardiovascular disease, larger clinical studies with systemic arginase inhibition are clearly warranted. Pharmacological interference with arginase holds great promise not only for the treatment of cardiovascular conditions, but also of cancer, autoimmunity, and unwanted immunosuppression, as reviewed in detail elsewhere. Several pharmacological inhibitors are available for experimental investigations. These belong to two main classes: boronic acids and analogues of N⁴-hydroxy-L-arginine. An important limitation of the currently available arginase inhibitors is that they show little or no selectivity for either of the arginase isoforms. For this reason, it remains unclear which isoform should be targeted to achieve the most beneficial effects. Since both arginase I and arginase II are expressed in the vasculature, isoform-specific arginase inhibitors are needed to fully explore the biological importance of the two isoforms.

Theoretically, arginase inhibition may be associated with side effects, particularly considering the key role arginase plays in ammonia detoxification in the urea cycle. However, arginase expression and activity in the liver is several-fold higher than in the vasculature and it is therefore unlikely that clinically relevant doses of inhibitors would suppress hepatic arginase to a degree that would impair the urea cycle. This notion is supported by the lack of toxic effects following long-term pharmacological arginase inhibition in animal models of hypertension and atherosclerosis. Moreover, long-term arginase inhibition apparently does not induce a compensatory up-regulation of the enzyme.

13. Conclusions

Available data clearly suggest that increased activity of arginase is of importance for several pathological changes associated with cardiovascular diseases. The effects seem to be exerted mainly via interference with NO bioavailability by limiting L-arginine sources and contributing to oxidative stress. Arginase therefore presents an attractive and promising pharmacological target in order to reverse the ‘arginase steal phenomenon’, enhance NO production, and limit oxidative stress (Figure 2). These effects of arginase inhibition have great potential against several cardiovascular diseases described in the present review. Although most current knowledge regarding arginase and the therapeutic effects of arginase inhibition are based on the data from studies in vitro and in experimental animals, results obtained in initial clinical studies support a role for arginase also in human disease.

Acknowledgements

Ms Helena Pernow is acknowledged for the illustration.

Conflict of interest: none declared.

Funding

The authors own work was supported by Swedish Research Council Medicine, the Swedish Heart and Lung Foundation, the European Foundation for the Study of Diabetes, the Stockholm County Council (ALF), Karolinska Institutet/Stockholm County Council Cardiovascular Programme, Novo Nordisk Foundation and Gustav V and Queen Victoria Foundation.

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